THE CILIATES OF STRONGYLOCENTROTUS DRÖBACHIENSIS: INCIDENCE, DISTRIBUTION IN THE HOST, AND DIVISION

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Most species of sea urchins harbor in their alimentary tracts a characteristic fauna of ciliated protozoa. As Kirby (1941) points out, these ciliates appear to be obligatory inquilines; at least there is no satisfactory evidence that they are normally free-living forms which become established accidentally in sea urchins. Although their occurrence is well known, their general biology has been only meagerly explored. Little is known regarding their host-specificity, mode of transmission, and affinities; their taxonomy is confused, and their structure and division are inadequately described. In fact, it appears that representatives of only three genera have been subjected to critical morphological examination. These are *Entorhipidium* and *Lechriopyla*, which were studied by Lynch (1929, 1930) with special reference to ciliation, cytoplasmic inclusions and the neuromotor system, and *Entodiscus*, which was similarly studied by Powers (1933b). The conjugation of only one of them, *Cryptochilidium echini*, has been described (Daïn, 1930), and none have been cultured. Thus the ciliates stand in need of much additional investigation.

The present study is a contribution to the general biology of the ciliates of *Strongylocentrotus dröbachiensis*, the north-ranging "green urchin" common to both coasts of this continent and to Europe. The study makes no claim to comprehensiveness, but is restricted to the aspects enumerated in the title.

The studies of Powers (1933a) have shown that in the Bay of Fundy region at least seven species of ciliates occur in the digestive tract of this urchin. Some of them are of invariable occurrence; others are found erratically. The seven ciliates and their present taxonomic status are as follows: (1) *Entodiscus borealis* (Hentschel, 1924). This large, common ciliate was first described by Hentschel under the name *Cryptochilum boreale* from specimens taken from *Echinus esculentus* in the Shetland Islands. Madsen (1931) also found it in *S. dröbachiensis* at Frederikshavn, Denmark. He removed it from *Cryptochilum* and created the genus *Entodiscus* to receive it. (2) *Madsenia indomita* (Madsen, 1931). This slender, abundant ciliate was first described by Madsen as *Entodiscus indomitus*. Kahl (1934) removed it from this genus and established the genus *Madsenia* to receive it. (3) *Biggaria gracilis* (Powers, 1933). This ciliate was described by Powers under the name *Cryptochilidium gracile*. Kahl transferred it to the genus *Biggaria*, which he founded in 1934. This ciliate and the aforementioned two are trichostomatous holotrichs belonging to the Entorhipidiidae, according to Kahl (1934). (4) *Plagiopyla minuta* Powers, 1933. This uncommon trichostomatous holotrich is referable to the Plagiopylidae. (5) *Cyclidium stercoris* Powers, 1935. This small hymenostomatous holotrich, a member of the Pleuronematidae, was first de-

scribed by Powers (1933a) as *Uronema sociale*. In 1935 he found this name to be invalid, being a homonym of *Uronema sociale* Penard, 1922. Since a further study of the ciliate convinced Powers of its close relationship to *Cyclidium,* he renamed it *Cyclidium stercoris.* (6, 7) *Euplotes* sp. and *Trichodina* sp. These two ciliates, a hypotrich and a peritrich, respectively, were not identified by Powers as to species. They were of uncommon occurrence.

The present study concerns these same ciliates—in particular, five of them—as found in 182 urchins from Frenchman's Bay, which to all practical purposes is a part of the Bay of Fundy. The urchins were collected three or four at a time almost daily throughout July and August 1947, and were examined immediately in an effort to discover the normal distribution of the ciliates. As a rule the examination of the first urchin of each small collection was well under way within 10 minutes after removal from its natural habitat.

The method employed by Lynch (1929) and Powers (1933a) in opening their urchins (cutting around the equator of the test and removing the intestine to a dish) was found to be unsuited to the present objectives, and the following method was adopted as least injurious to the alimentary tract. The urchin is rinsed in running tap water to remove external free-living ciliates and is laid oral surface uppermost on the table. With strong, sharply pointed scissors a circular cut is made around the test about 5 mm. to the outside of its oral opening. The central piece, consisting chiefly of lantern, peristomial membrane and adjacent, excised ring of the test, is lifted out and detached by severing the esophagus. With sharply pointed forceps or small scissors the mesenteries which hold the stomach (inferior or oral spiral of the alimentary tract) to the oral surface of the test are severed, and the opening in the test is enlarged by making a second circular cut about 5 mm. to the outside of the first. The perivisceral fluid is pipetted out. Thus the entire alimentary tract is left in its normal position in the test. With good fortune the stomach is uninjured, though sometimes its oral wall is slit in one or two places. The intestine (superior or aboral spiral of the alimentary tract) and the rectum (short, straight aboral portion which runs more or less meridionally to the anus) are never injured. With small scissors incisions large enough to admit an ordinary pipette are made at intervals in the alimentary tract and samples of the enteric fluid are removed. These may be examined at once or spread out on cover slips for fixation and subsequent staining.

The ciliates of *S. dröbachiensis* are found chiefly in the rather abundant enteric fluid, though if the food pellets are of a loose texture, owing to the ingestion of filamentous algae, they occur in limited numbers within the pellets. I have never found them in the siphon, in the perivisceral fluid or elsewhere in the urchin. André (1910) states that he found *Euplotes charon* on the exterior and in the perivisceral fluid of *Echinus esculentus,* and *Cryptochilidium echini* in abundance in both the intestine and perivisceral fluid of *Echinus miliaris* and *Paracentrotus lividus.* Hentschel is of the opinion that these urchins were moribund or that the ciliates were introduced into the perivisceral fluid in opening the urchins. To open an urchin with the assurance that the thin-walled and sinuous alimentary tract is still quite intact so that no ciliates can escape into the surrounding fluid, requires not only extreme care but also a very thorough examination of the condition of the exposed alimentary tract. Whether André's urchins received the requisite

care and subsequent examination is not clear. Lacking the assurance that these precautions were observed, I am disposed to doubt that the ciliates occurred normally in some of the sites in which he found them.

Incidence of Infection and Distribution in the Host

Of the 182 urchins, whose tests varied in diameter from 3 cm. to 6 cm., all were infected with *E. borealis* and *M. indomita;* 181 were infected with *B. gracilis;* 98 with *C. stercoris;* 28 with *P. minuta;* 24 with *Euplotes;* and four with *Trichodina.* These observations appear to be in general agreement with Powers' findings which were made 17 years earlier, though Powers (1933a) gives no actual figures on incidence of infection, except for *P. minuta* which occurred in 10 per cent of the hosts. The present incidence of infection with this ciliate amounts to 15.4 per cent. Powers states that *E. borealis, M. indomita* and *C. stercoris* occurred "in great abundance," and that infection with *B. gracilis* seemed "universal." Presumably the percentage of hosts infected with *Euplotes* and *Trichodina* was small.

The total number of ciliates present in a specimen of *S. dröbachiensis* from Frenchman's Bay is unbelievably great. Evidently Madsen's specimens from Denmark likewise harbored intense infections, for he speaks of the ciliates as occurring "in ungeheuren Mengen." To obtain a reasonably accurate idea of the general intensity of infection, a number of ciliate counts were made in the present study. The number of individuals of each species was counted in seven samples of fluid from different regions of the digestive tract of each of 12 urchins. These samples were taken from the first, middle and last thirds of the stomach; from corresponding regions of the intestine; and from the rectum (one sample). Based on the experience gained from these counts, estimates were made of the intensity of infection in seven samples from each of the remaining 170 urchins. Thus the findings on the intensity of infection in different regions of the alimentary tract are admittedly estimates for 170 of the 182 urchins, but it is believed that these estimates are reasonably faithful representations of the actual facts. The examination of these samples also served to disclose any differential distribution of the ciliates which might prevail along the course of the digestive tract. The degrees of infection were classified somewhat arbitrarily as "heavy," "moderate," or "light." The term "heavy," as applied to a particular species of ciliate, means an infection amounting to 500–1000 or more individuals per 0.1 cc. of enteric fluid; "moderate" indicates an infection of 50–500 per 0.1 cc.; and "light," fewer than 50 per 0.1 cc.

In the following summary of the results of these examinations, the various species will be considered separately, beginning with *E. borealis,* which was found in every urchin. The stomach was infected heavily in 40 urchins, moderately in 126, and lightly in 16; the intestine, heavily in seven urchins, moderately in 59, and lightly in 116; the rectum was lightly infected in all of them. Thus it is evident that *E. borealis* was most abundant in the stomach, less abundant in the intestine, and of relatively scant occurrence in the rectum. The principal or preferred site of infection of *E. borealis* is clearly the stomach.

Considering *M. indomita,* which was also found in every urchin, none of the 182 revealed heavy infections of the stomach, though 18 of them showed moderate infections and 24, light infections. As to the intestine, 131 urchins showed heavy infections; 39, moderate infections; and 12, light infections. The rectum was always

lightly infected. Thus *M. indomita* is primarily an inhabitant of the intestine. It may occur in the stomach in moderate numbers, and it occurs in the rectum in much reduced numbers.

Turning to *B. gracilis,* a very different type of distribution was encountered. This ciliate was never found in the stomach. It occurred as light infections in the intestine of only 16 urchins, though in these it was restricted to the last pouch of the intestine. However, it occurred as infections of moderate intensity in the rectum of 178, and as light infections in three. (Only one was uninfected.) Thus *B. gracilis* is clearly an inhabitant of the rectum, from which it may extend forward (orally) into the terminal intestinal pouch.

C. stercoris, present in 98 urchins, was seen in the stomach of only one, and then as a light infection. Eighteen urchins harbored heavy intestinal infections; 61, moderate infections; and 19, light infections. The rectum was lightly infected in all 98. Hence, *C. stercoris* is primarily an inhabitant of the intestine.

P. minuta occurred as light infections in the intestine and rectum of 28 urchins. In many of these urchins it was distinctly more abundant than in those examined by Powers, since he never found more than 12 specimens per host. Many of the present samples contained 10 to 20 specimens, and a single sample represents only a small portion of the enteric fluid. Hence, many of the urchins contained hundreds of specimens of this ciliate.

Euplotes likewise occurred only as light infections of the intestine and rectum. The samples of fluid from the 24 urchins which were infected usually contained only five to ten specimens per sample.

Trichodina was found in only four urchins, as extremely light infections of the intestine and rectum. Only one or two specimens per sample were found in these hosts.

These results show conclusively that the various kinds of ciliates are not distributed indifferently along the digestive tract but exhibit to some extent definite preferences for different regions. Actually the three regions under consideration do not have precise anatomical limits, but rather pass insensibly one into another. To what extent the regions differ physiologically is not entirely clear. It seems to be generally assumed that the entire alimentary tract participates in the functions of digestion, absorption, and transport of dissolved substances to the perivisceral fluid. However, Weese (1926) found that an extract of the stomach of *S. dröbachiensis* had a pH of 6.3, whereas a similar extract of the intestine had a pH of 6.6, and it has long been known that in *Echinus esculentus* glandular cells are more abundant in the stomach than elsewhere (Chadwick, 1900). Thus minor physiological and histological differences occur along the digestive tract, and the physiological differences are probably greater than the limited evidence indicates. At least the differential distribution of the ciliates points to such differences, for some of them are adapted to life in one region, some in another. *B. gracilis* shows the highest degree of selectivity in its choice of a habitat, for it is almost exclusively an inhabitant of the rectum. Whether the nature of the rectal flora, on which it presumably feeds, or the chemical and physical properties of the rectal fluid account for its localization is not clear. Only *E. borealis* occurs regularly in the stomach. Evidently it has a high degree of tolerance for the digestive juices of this region. The remaining five ciliates show a distinct preference for the in-

testine. Since the ciliates of all sea urchins escape regularly with the feces (apparently never as cysts, but in their usual trophic form), any ciliate which occurs in the stomach or intestine will also be found in the rectum. Hence, all seven species inhabiting *S. dröbachiensis* are found in this region. Powers (1933a) showed that the ciliates of sea urchins have considerable tolerance for sea water, and it is assumed that they are ingested in the trophic form by new hosts. However, sea urchins regularly engage in cannibalism, small, weak or injured specimens being readily consumed, even to the spines and test. Cannibalistic practices afford an obvious means of transferring the ciliates from one urchin to another.

It is important to mention that the foregoing distribution prevails only in well-fed urchins and that all the 182 urchins appeared to be reasonably well-fed. The stomach of each of them contained scores or even hundreds of food pellets, which consisted of algae, small specimens of the clam *Mya arenaria*, and unrecognizable debris. When urchins were kept in laboratory aquaria without food for a week, the stomach was always found to be empty of food and practically devoid of ciliates, whereas the intestine always contained many food pellets and ciliates. (Such urchins, if kept together, must be uninjured and fairly uniform in size; otherwise cannibalism develops.) When other urchins were kept without food for two or three weeks, the intestine, as well as the stomach, was found to be empty, and the food pellets and ciliates were restricted to the rectum, the ciliates in much reduced numbers. Thus the food pellets and the ciliates simultaneously shift aborally when food is withheld.

Periodicity of Division

Upon examining a dozen or more urchins in the early part of July, I was especially impressed by two seemingly contradictory aspects of the occurrence of *E. borealis, M. indomita* and *C. stercoris:* first, their great abundance, and secondly, the absence of dividing specimens in populations of such density. *Trichodina, Euplotes* and *P. minuta* are excluded from immediate consideration, for they were found in such limited numbers that the probability of observing divisional stages was somewhat remote. *B. gracilis* is likewise excluded for the present, since it was found in division in practically every urchin. The absence of dividing individuals in the remaining three species was genuinely puzzling, and the search for them was intensified. Throughout July and August more urchins were collected, as usual at low tide, and examined immediately, not only during the day but at night as well, so that finally practically all the hours of the day and night were represented in these examinations. The findings with respect to division will be presented for each of the three species in turn, beginning with *E. borealis.*

Of 88 urchins collected in July, only one, a specimen taken at 2:00 A.M., July 27, showed *E. borealis* in division, though rather sparingly. Six smear preparations of the stomach contents of this urchin were fixed on cover glasses in Schaudinn's fluid and stained in Mayer's acid hemalum. These revealed some 2400 specimens of *E. borealis*, only 3 of which were dividing. Thus 88 urchins which undoubtedly contained many hundreds of thousands of individuals furnished an utterly insignificant number of dividing specimens. Of 94 urchins collected in August, five contained *E. borealis* in division. In three of four urchins collected at 10:00 A.M., August 9, it was dividing in great abundance both in the stomach and first third of

the intestine. So plentiful were the dividing forms that every sample of 0.1 cc., when examined under the dissecting binocular, was seen to contain 25–50 individuals in division, and smears made of material from these hosts showed all the stages in the division process. Again on August 10, two of four urchins contained dividing specimens, though not plentifully. These were the last specimens seen in division, though daily examinations were continued throughout August.

These results indicate that division in *E. borealis* is a cyclical phenomenon—that short periods of intense divisional activity alternate with long periods of non-divisional life. The factors which account for this apparent rhythmicity in the reproductive activities of the ciliate are at present unknown. Perhaps a more extensive study of the entire urchin-ciliate relationship—preferably a study embracing the entire year—would supply an explanation. The ability of *E. borealis* to maintain itself in such great numbers in the absence of frequent divisions indicates a low death-rate in the alimentary tract and a low percentage of losses at defecation. (Counts of ciliates expelled with the feces, to be mentioned shortly, support the latter conclusion.)

Powers (1933b) also had difficulty in finding dividing specimens of *E. borealis,* and indeed found only three in all the living specimens examined. On the other hand, Hentschel, in a study of *E. borealis* from *Echinus esculentus,* does not mention any difficulty in finding dividing specimens in heavily infected urchins, though he had only three such urchins. However, the relation of *E. borealis* to *Echinus esculentus* is evidently different from its relation to *S. dröbachiensis,* since Hentschel found only ten infected individuals among 52 urchins, and seven of these were lightly infected. Powers (1933a) remarks that *E. borealis* seems to be normally associated with *S. dröbachiensis,* "but is able to infest *E. esculentus* when the two species of sea urchins inhabit the same locality."

The task of finding *M. indomita* in division was even more arduous and unrewarding than the preceding, in spite of the fact that this ciliate, as in Madsen's specimens of *S. dröbachiensis,* was far more abundant than *E. borealis.* Only one dividing specimen was seen in 88 urchins in July, although some 450 samples containing *M. indomita* were examined and 52 stained cover-slip preparations of additional samples were studied. Many of the fresh samples contained well over 1000 specimens and many of the stained slides had 300 specimens per cover slip. A second dividing individual was seen August 9. Finally, three of seven urchins examined on August 28–29 showed *M. indomita* in division in considerable numbers, and slides made on these two days sufficed to show all the stages of division. Thus *M. indomita* is also able to maintain itself in immense numbers for long periods in the absence of division. Again, this circumstance indicates that it is not lost in great numbers with the feces and that it does not perish readily in the intestine. When division finally occurs, it appears to assume the character of a "mitotic flare," affecting great numbers of individuals simultaneously in any particular urchin. Neither Powers nor Madsen mentions the division of *M. indomita.*

As has been said, only 18 of the 182 urchins harbored infections of *C. stercoris* which qualified as "heavy." However, some of these infections were extremely heavy, each sample containing a veritable swarm of cyclidia, and there were 61 "moderate" infections. Hence, this ciliate was present, both in fresh samples and stained slides, in sufficient numbers to reveal divisional stages had they been

present. Nevertheless, not a specimen could be found in division in July. Then, for a period of six days, beginning August 11, *C. stercoris* was seen in division in nearly every urchin examined—in 19 out of 20, to be exact. However, the number of dividing specimens per urchin was small and not all the stages of division could be found. This outbreak of division appeared to subside on August 16, and no more dividing specimens could be found. Thus division in *C. stercoris* appears to be cyclical, or at least sporadic.

Finally, a word concerning the division of *P. minuta* and *B. gracilis* must be appended. Infections with *P. minuta* were always light, and it was found in only 28 urchins. Few examples of division could be expected in such light infections, and none were found in 27 of the urchins. Then, on August 25, one urchin disclosed a dividing specimen. This urchin was subjected to a very thorough examination, and a total of 12 dividing individuals were found in 20 samples of rectal and intestinal contents. Hence, such evidence as is available indicates that the division of *P. minuta* also assumes a rhythmic character. *B. gracilis,* on the contrary, could be found in division readily in any urchin and therefore gave no evidence of major rhythms which affect the entire population of an urchin. However, *B. gracilis,* unlike all the other ciliates, is predominantly a rectal inhabitant. Counts of the ciliates lost at defecation, to be discussed immediately, showed that it is lost in greater numbers than any other ciliate. Therefore, it would seem that it must remain in a state of constant division in order to maintain itself within its host.

Ciliate Losses Accompanying Defecation

It is generally implied that the ciliates of sea urchins are lost in considerable numbers with the feces, yet I have found no reliable numerical estimates of these losses. An attempt was therefore made to arrive at an estimate by counting the ciliates which accompanied the escape of the fecal material. The feces of *S. dröbachiensis* are passed in the form of fairly firm, more or less spherical pellets which measure 1.0–1.5 mm. in diameter. The pellets are passed singly and never in rapid succession; each emerges very slowly. Hence, the amount of rectal fluid which is lost at defecation seems to be reduced to the minimum. The pellets themselves contain no ciliates. Thus the ciliates which are actually lost must slip out between the wall of the anal canal and the surface of the escaping pellet. It is evident that the nature of the pellets and the mechanism of defecation do not facilitate the loss of ciliates.

The estimates of ciliate losses were arrived at by collecting and examining not only the pellets themselves but also the sea water in the immediate vicinity of the emerging pellets. A freshly collected urchin was placed in a dish of sea water in which it could move about and defecate normally. When a pellet first made its appearance among the anal plates, the end of a pipette was brought quite near it and 1 cc. of sea water was taken up and transferred to a watch glass for examination. A second cc., or a third or fourth, was taken up as the pellet continued its outward progress; then the pellet itself plus an additional cc., and a final cc. after emergence, were removed. Thus, it was hoped, all the escaping ciliates might be captured for counting. If an urchin failed to defecate within a reasonable time, it was given a piece of clam to feed on; ingestion usually stimulated defecation. When

an urchin was kept under observation longer than 30 minutes, the sea water was changed, or better, the dish was set in an aquarium table and a gentle stream of sea water was directed into it. Urchins soon become sluggish in the absence of ample oxygen. Seven urchins, each of which was kept under observation for 1 hour, expelled a total of 186 pellets, and a total of 84 ciliates, which were distributed as follows: *E. borealis,* 16 specimens; *M. indomita,* 18; *C. stercoris,* 16; *B. gracilis,* 33; *P. minuta,* one. The results indicate, therefore, that approximately one ciliate is lost per two fecal pellets and that on the average an urchin loses 12 ciliates hourly or 288 daily. A number of additional counts, based on observational periods of 20–30 minutes each, supported these conclusions. A daily loss of 288 ciliates per urchin, or a loss of twice or thrice this figure should pellets be passed in appreciably greater numbers, is relatively small, for a single drop (about 0.05 cc.) of enteric fluid may contain as many as 1000 ciliates. Bearing in mind that a single division doubles the number of ciliates, it is evident that infrequent divisions would suffice to compensate for the losses which accompany defecation.

B. gracilis was lost in greater numbers than any of its confreres, although it is by no means as plentiful in the alimentary tract as *E. borealis* and *M. indomita.* Its relatively severe losses are evidently correlated with its habitat, since it is the only strictly rectal ciliate of the entire fauna, and indeed extends quite to the anal opening. In this disadvantageous position, it is lost in significant numbers, and as a consequence must remain in a state of constant division, it would seem, in order to maintain itself.

Method of Division

Entodiscus borealis. At division the single spherical or ovoid macronucleus elongates, and the micronucleus separates into halves with the production of a fairly long interconnecting strand (Fig. 1). The macronucleus then constricts cleanly into halves while the daughter micronuclei rest at opposite ends of the cell (Fig. 2). Each daughter macronucleus becomes spherical, and the posterior micronucleus begins to migrate to the usual position near the anterior surface of the macronucleus (Fig. 3). Thus division, as Hentschel remarks, "exhibits no remarkable features," but since he gives no figures of the process, three are included here.

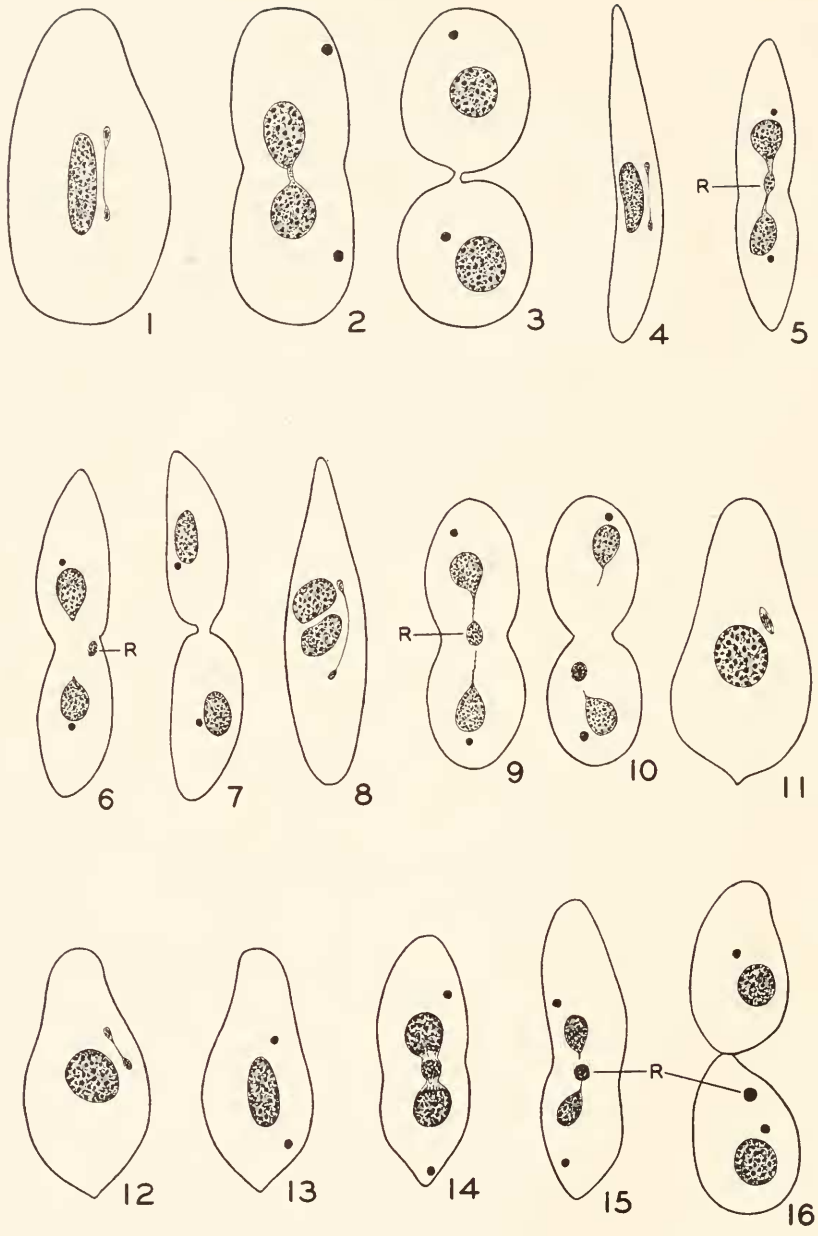
Although the vast majority of the non-dividing specimens showed a single macronucleus, a limited number, as in those examined by Powers (1933b), contained a macronucleus which consisted of 2–7 parts, but always accompanied by a single resting micronucleus. In any particular individual of this type the parts were of approximately equal size, but the more parts per specimen, the smaller their size. Powers diagrams these multipartite macronuclei and gives measurements of their parts.

Issel (1903) observed that the macronucleus of various species of *Ancistruma* from marine pelecypods may be seemingly fragmented, often into 2–7 parts. Uyemura (1934) noted that the macronucleus of *Entorhipidium fukuii* from various urchins may be multipartite (in 2–8 pieces) and may be accompanied by two micronuclei, whereas there is typically only one. Uyemura concluded that nuclear conditions of this sort must be intimately related to cell division, though no dividing specimens could be found. Finally, Yagiu (1935), in a study of *Cryptochilidium ozakii* from Japanese urchins, likewise noted that the macronucleus may

be in one part or in 2–7, but always accompanied by a single resting micronucleus.

Powers (1933b) interprets the multipartite condition in terms of a nuclear reorganization, and Yagiu regards the macronuclear elements as daughter macronuclei, since some of the large single macronuclei were lobed as if separating into smaller parts, but neither investigator seems genuinely satisfied with his own interpretation. However, the studies of Kidder (1933a,b) undoubtedly furnish the correct explanation of Issel's findings, and supply the probable explanation of the corresponding macronuclear conditions in *Entorhipidium fukuii, Cryptochilidium ozakii* and *E. borealis.* According to Kidder, the 2–7 macronuclei of *Ancistruma* are not actually fragments of a former macronucleus but are the macronuclear anlagen of a reorganizing exconjugant. In *A. isseli* from the mantle cavity of *Modiola modiolus,* Kidder (1933b) finds that the amphinucleus (synkaryon) of the exconjugant undergoes three divisions, from the products of which one micronucleus and seven macronuclear anlagen are derived. Three micronuclear divisions and three accompanying cell divisions follow. At these cell divisions the anlagen are segregated into the daughter cells as follows: at the first division, four into one daughter and three into the other; at the second division, two into each of three daughters and one into the fourth daughter which now has the typical nuclear complex and does not divide again; at the third division, one into each of six daughters. Thus specimens having one, two, three, four, or seven macronuclear parts may be found in a normal population of *A. isseli.* Although conjugation has not been reported in *E. borealis, C. ozakii* or *Entorhipidium fukuii,* it is logical to assume that it occurs, and reasonable to assume first, that it follows the pattern of *A. isseli,* and secondly, that the apparently multimacronucleate individuals are exconjugants which have not completed their reorganizational divisions. The schema outlined for *A. isseli* does not call for specimens with quinquepartite or sexpartite macronuclei, and these actually are almost non-existent in *E. borealis.* In some 5700 specimens Powers (1933b) found 162 which had multipartite macronuclei; they fell into these categories: bipartite, 103; tri-, 34; quadri-, 21; quinque-, O; sex-, 1; septem-, 3. In 4700 specimens from July and August collections I found 122 with multipartite macronuclei, distributed as follows: bi-, 79; tri-, 16; quadri-, 21; quinque-, O; sex-, one; septem-, five. No specimen in either collection had a macronucleus of more than seven parts. In accordance with expectations, if it be assumed that these specimens were exconjugants, bipartites predominated in both collections; three- and four-partites were plentiful and occurred in approximately equal numbers; five- and six-partites were either absent or so rare that they may be looked on as atypical; and seven-partites were the least common of all the expected types. If these are actually exconjugants, one would expect to find occasionally a specimen with a dividing micronucleus, yet neither Powers nor I could find even one. It is hoped that a further study of the ciliate will disclose the conjugants themselves and thereby show conclusively whether the present explanation is correct. In *Cryptochilidium echini* Daïn found that the synkaryon undergoes two divisions, thus producing one micronucleus and three macronuclear anlagen. Two segregation divisions follow, so that specimens having tri- or bipartite macronuclei occur normally.

Madsenia indomita. This slender ciliate contains a single, slightly elongated macronucleus and one micronucleus which usually lies just posterior to the macro-



EXPLANATION OF FIGURES

FIGURES 1–16. Division in four species of ciliates from the alimentary tract of *Strongylocentrotus dröbachiensis*. Schaudinn fixation; stain, Mayer's acid hemalum; camera lucida.

FIGURES 1–3. *Entodiscus borealis:* 1, macronucleus elongating, micronucleus in telophase; 2, division of macronucleus; 3, separation of daughters. × 225.

nucleus. At division the macronucleus elongates and the micronucleus divides typically (Fig. 4). The daughter micronuclei migrate to opposite poles of the macronucleus (Fig. 5), and the latter begins to constrict into halves. However, as the halves of the macronucleus draw apart, a small mass of macronuclear material remains as a residuum midway between the halves and within the attenuated nuclear membrane (Fig. 5). When the daughter macronuclei separate, the residual mass, which consists of little more than six to eight deeply staining granules in a lightly staining matrix, remains behind in the cytoplasm (Fig. 6). The life of the residual mass is brief, for it is resorbed before the daughter cells separate (Fig. 7). The elimination of a portion of the macronuclear substance at division is not of invariable occurrence; in some specimens the macronucleus divides cleanly with no suggestion of a residuum.

The accumulation of a mass of macronuclear material in the elongated nuclear membrane, its subsequent abandonment by the dividing macronucleus, and its final resorption in the cytoplasm have been described in four species of *Conchophthirius* (Kidder, 1933c, 1934) and in *Ancistruma isseli* (Kidder, 1933a) from pelecypods, and in *Cryptochilidium minor* from sea urchins (Yagiu, 1934). Any such material which in different ciliates is expelled by a variety of means from the macronucleus is usually looked on as effete and unwanted, and the process is interpreted as having a salutary effect on the macronucleus.

A very small percentage of the specimens of *M. indomita* contained a bi- or tripartite macronucleus, the parts being arranged usually in a series along the longitudinal axis of the ciliate. In two specimens which had a bipartite macronucleus, the micronucleus was dividing. One such specimen is shown in Figure 8. Perhaps these specimens were exconjugants undergoing their final reorganizational division.

Cyclidium stercoris. This is the smallest of the ciliates which occur abundantly in *S. dröbachiensis.* It has a spherical macronucleus which lies well toward the anterior end of the organism. The single micronucleus is usually found near the anterior surface of the macronucleus, but it may be at the side of, or just posterior to, the macronucleus. The scarcity of dividing specimens has been mentioned, and the early divisional stages were so elusive that none could be found in 32 stained cover-glass preparations which were made August 11–16. These preparations showed a number of specimens in the late stages of division and sufficed to demonstrate conclusively that the dividing macronucleus always discards a portion of its substance in the form of a residual mass within the attenuated macronuclear membrane. In Figure 9 the residual mass still occupies the mid-

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FIGURES 4–7. *Madsenia indomita:* 4, macronucleus elongating, micronucleus in telophase; 5, division of macronucleus with small mass of residual chromatin (R) at center of elongated nuclear membrane; 6, residual mass undergoing resorption; 7, separation of daughters. × 475.

FIGURE 8. *Madsenia indomita.* Micronuclear division in specimen with bipartite macronucleus. Probably the final reorganizational division of an exconjugant. × 475.

FIGURES 9–10. *Cyclidium stercoris:* 9, division of macronucleus with elimination of a conspicuous mass (R) of macronuclear chromatin; 10, residual mass free in cytoplasm. × 735.

FIGURES 11–16. *Biggaria gracilis:* 11, macronucleus spherical, micronucleus in metaphase; 12, micronucleus in telophase; 13, macronucleus elongating; 14, accumulation of residual macronuclear chromatin at center of dividing macronucleus; 15, residual mass (R) free in cytoplasm; 16, residual mass being resorbed, always in posterior organism. × 335.

point of the membrane, whereas in Figure 10 it is detached. The macronuclear behavior differs from that of *Cyclidium ozakii,* a ciliate of the echinoid *Anthocidaris crassispina,* for in *C. ozakii,* according to Yagiu (1933), the separation of the daughter halves is clean. However, even congeneric species are known to differ with respect to the extrusion of macronuclear material at division. Thus the dividing macronucleus of *Ancistruma mytili* never discards any of its substance, whereas the macronucleus of *A. isseli,* as has been said, always casts out a substantial residual mass (Kidder, 1933a).

Biggaria gracilis. The division of this striking ciliate, whose anterior half is distinctly flattened dorso-ventrally (using this term in a physiological sense) and whose posterior half is somewhat spherical and bulbous, offered no difficulty in view of the abundance of dividing forms. The macronucleus usually has the shape of an ellipsoid and lies transversely at the center of the organism. The single micronucleus is found just anterior to the macronucleus. With the approach of division the macronucleus becomes spherical and the micronucleus assumes a position antero-lateral to the macronucleus. In this location the micronucleus elongates and becomes spindle-shaped (Fig. 11). Then, with its long axis directed obliquely across the cell, the micronucleus completes its division (Fig. 12). I have examined at least 20 specimens in which the micronucleus was in various stages of division, and without exception it occupied the position just described. Thus the dividing micronucleus does not lie at the side of the macronucleus in the future cleavage plane of the cytoplasm, nor does the macronucleus begin to elongate until the micronucleus has completed its division. (In these aspects of nuclear behavior *B. gracilis* agrees with *A. isseli,* according to the figures of Kidder, 1933a.) The daughter micronuclei now move toward opposite ends of the cell, and the macronucleus begins to elongate (Fig. 13). Soon the macronucleus develops its divisional constriction, but in so doing a conspicuous mass of macronuclear material accumulates precisely at the center of the constriction (Fig. 14). This mass is of course the residual chromatin and associated substances which will be discarded when the macronuclear halves separate. A slight constriction appears in the cytoplasm at this stage. The daughter macronuclei now draw apart (Fig. 15), leaving the residual mass behind, while the cytoplasmic constriction continues to deepen. Finally, the residual mass passes into the posterior daughter cell where its resorption occurs, the posterior micronucleus migrates to its normal resting position just anterior to its macronucleus (Fig. 16), and the cells separate. The resorption of the residual mass is completed after the separation of the daughter cells. I have examined some 25 specimens in the final stages of division (Fig. 16), and without exception the residual mass was to be found in the posterior cell. In the four species of *Conchophthirius* and in *A. isseli* in which this type of macronuclear reorganization occurs the residual mass may be resorbed in either daughter cell, according to Kidder (1933a, c; 1934).

Plagiopyla minuta. Of the 12 dividing specimens which were seen, seven were successfully affixed to cover slips and stained. Unfortunately, Mayer's acid hemalum proved to be unsuited to the staining of *P. minuta,* since it stains the cytoplasm and food vacuoles of this ciliate intensely, thereby obscuring the nuclei. Owing to a scarcity of material, the Feulgen method could not be employed, and a consideration of division in *P. minuta* must be deferred. When ap-

plied to the remaining ciliates, acid hemalum gave excellent results, leaving the nuclei well stained against a faintly tinted background.

Summary

Of 182 specimens of *S. dröbachiensis,* all were infected with *Entodiscus borealis* and *Madsenia indomita*; 181 with *Biggaria gracilis*; 98 with *Cyclidium stercoris;* 28 with *Plagiopyla minuta;* 24 with *Euplotes* sp.; and four with *Trichodina* sp.

E. borealis occurs primarily in the stomach, though it extends into the intestine and rectum; *B. gracilis,* almost exclusively in the rectum; the five remaining species, chiefly in the intestine, less commonly in the rectum.

Although *E. borealis, M. indomita* and *C. stercoris* were present in immense numbers, they were rarely found in division. The evidence indicates that division in these ciliates, and probably in *P. minuta,* is a cyclical phenomenon; short periods of intense divisional activity appear to alternate with long periods of non-divisional life. *B. gracilis,* to the contrary, was dividing in nearly every urchin.

The ability of some of the ciliates to maintain themselves in enormous numbers in the absence of frequent divisions indicates that they do not perish readily in the alimentary tract and that they are not lost in great numbers at defecation. Counts of discharged fecal pellets and escaping ciliates indicate that an average of only one ciliate is lost per two fecal pellets. This rate of loss is low, in view of the great number of ciliates present per urchin. Thus infrequent divisions suffice to compensate for these moderate losses. *B. gracilis* is lost in greater numbers than any other species and must divide constantly in order to maintain itself.

At each division of *B. gracilis* and *C. stercoris* a mass of macronuclear material aggregates at the center of the elongated macronuclear membrane. This mass is discarded into the cytoplasm when the daughter macronuclei separate. A similar macronuclear reorganization usually accompanies the division of *M. indomita.*

Literature Cited

André, E., 1910. Sur quelques infusoires marins. *Rev. Suisse de Zool.,* **18**: 173–187.

Chadwick, H. C., 1900. Echinus. *Liverpool Marine Biology Memoirs,* III.

Daïn, Lydia, 1930. Die Conjugation von Cryptochilum echini Maupas. *Arch. f. Protistenk.,* **70**: 192–216.

Hentschel, C. C., 1924. On a new ciliate, Cryptochilum boreale nov. sp., from the intestine of Echinus esculentus Linn., together with some notes on the ciliates of echinoids. *Parasitol.,* **16**: 321–328.

Issel, R., 1903. Ancistridi del Golfo di Napoli. *Mitt. Zool. Sta. Neapel,* **16**: 63–108.

Kahl, A., 1934. Ciliata entocommensalia et parasitica. In Grimpe, G., und E. Wagler: *Die Tierwelt der Nord- und Ostsee.* Lief. 26, Teil II. C4, 147–183. Leipzig: Akademische Verlagsgesellschaft.

Kidder, G. W., 1933a. On the genus Ancistruma Strand (Ancistrum Maupas). I. The structure and division of A. mytili Quenn. and A. isseli Kahl. *Biol. Bull.,* **64**: 1–20.

Kidder, G. W., 1933b. On the genus Ancistruma Strand (= Ancistrum Maupas). II. The conjugation and nuclear reorganization of A. isseli Kahl. *Arch. f. Protistenk.,* **81**: 1–18.

Kidder, G. W., 1933c. Studies on Conchophthirius mytili DeMorgan. I. Morphology and division. *Arch. f. Protistenk.,* **79**: 1–24.

Kidder, G. W., 1934. Studies on the ciliates from fresh water mussels. II. The nuclei of Conchophthirius anodontae Stein, C. curtus Engl., and C. magna Kidder, during binary fission. *Biol. Bull.,* **66**: 286–303.

KIRBY, H., 1941. Relationships between certain protozoa and other animals. In Calkins, G. N., and F. M. Summers: *Protozoa in biological research,* 890–1008. New York: Columbia University Press.

LYNCH, J. E., 1929. Studies on the ciliates from the intestine of Strongylocentrotus. I. Entorhipidium gen. nov. *Univ. Calif. Publ. Zool.,* **33**: 27–56.

LYNCH, J. E., 1930. Studies on the ciliates from the intestine of Strongylocentrotus. II. Lechriopyla mystax gen. nov., sp. nov. *Univ. Calif. Publ. Zool.,* **33**: 307–350.

MADSEN, H., 1931. Bemerkungen über einige entozoische und freilebende marine Infusorien der Gattungen Uronema, Cyclidium, Cristigera, Aspidisca und Entodiscus gen. nov. *Zool. Anz.,* **96**: 99–112.

POWERS, P. B. A., 1933a. Studies on the ciliates from sea urchins. I. General taxonomy. *Biol. Bull.,* **65**: 106–121.

POWERS, P. B. A., 1933b. Studies on the ciliates from sea urchins. II. Entodiscus borealis (Hentschel), (Protozoa, Ciliata), behavior and morphology. *Biol. Bull.,* **65**: 122–136.

POWERS, P. B. A., 1935. Studies on the ciliates of sea-urchins. A general survey of the infestations occurring in Tortugas echinoids. *Carnegie Institution of Wash., Publ.* **452**: 293–326.

UYEMURA, M., 1934. Über einige neue Ciliaten aus dem Darmkanal von japanischen Echinoideen (I). *Sci. Rep. Tokyo Bunrika Daigaku,* Sec. B, **1**: 181–191.

WEESE, A. O., 1926. The food and digestive processes of Strongylocentrotus dröbachiensis. *Publ. Puget Sound Biol. Sta.,* **5**: 165–179.

YAGIU, R., 1933. Studies on the ciliates from the intestine of Anthocidaris crassispina (A. Agassiz). I. Cyclidium ozakii sp. nov. and Strombilidium rapulum sp. nov. *Jour. Sci. Hiroshima Univ.,* Sec. B, Div. I, **2**: 211–222.

YAGIU, R., 1934. Studies on the ciliates from the intestine of Anthocidaris crassispina (A. Agassiz). II. Cryptochilidium sigmoides sp. nov. and Cryptochilidium minor sp. n. *Jour. Sci. Hiroshima Univ.,* Sec. B, Div. I, **3**: 25–31.

YAGIU, R., 1935. Studies on the ciliates from sea urchins of Yaku Island, with a description of a new species, Cryptochilidium ozakii sp. nov. *Jour. Sci. Hiroshima Univ.,* Sec. B, Div. I, **3**: 139–147.